OXYGEN CONSUMPTION AND CARBON DIOXIDE ELIMINATION IN TETRAHYMENA GELEII FURGASON [1]

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Unicellular organisms make excellent material for the study of various cellular phenomena. *Tetrahymena geleii,* a colorless holotrichous ciliate, is an exceptionally desirable organism for such physiological studies, mainly because it is readily grown in rather simple, sterile organic media.

This organism contains cytochromes c, b, a, and possibly a_2 and its oxygen consumption is inhibited by cyanide and carbon monoxide (Baker and Baumberger, 1941). It grows well at ordinary oxygen tensions, but is most prolific in pure oxygen (Pace and Ireland, 1945). It soon dies in oxygen tensions below 10 mm. Hg partial pressure.

Many investigations have been carried out with *Tetrahymena* as the experimental organism. There is, however, much to be desired in respect to our knowledge concerning respiratory metabolism; knowledge that may lead to a better understanding of the respiratory mechanisms of cells in general. Since very little is known concerning oxygen consumption, carbon dioxide elimination, and respiratory quotient in *Tetrahymena,* the following investigations were made.

Material and Methods

The "W" strain of *Tetrahymena geleii,* kindly furnished by Professor George Kidder, used throughout these investigations, was grown in this laboratory in a 2 per cent proteose-peptone (Difco) solution. They grow very rapidly in this solution and usually reach maximum numbers within four to six days depending upon temperature and the number introduced.

A Barcroft-Warburg respirometer was used for ascertaining oxygen consumption and carbon dioxide elimination. Temperatures below room temperature were obtained in the bath by means of an Aminco refrigerating unit.[2]

Before each experiment the organisms were washed thoroughly in a buffered solution in which they were left during the experiment. This solution is one similar to that used by Pace and Belda (1944) for *Pelomyxa,* with slight modifications,[3] and was used for these tests chiefly because of the several advantages it has over the proteose-peptone solution. It is much simpler to work with since there is no

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[3] The buffer solution used for washing and testing *Tetrahymena geleii* in the Barcroft-Warburg respirometer contained the following: $K_2HPO_4 \cdot 3H_2O$, 65.5 mg.; $NaH_2PO_4 \cdot H_2O$, 40.0 mg.; $CaCl_2$, 100 mg.; $MgCl_2$, 2.0 mg.; and redistilled H_2O to 1,000 ml.

food material on which bacteria can grow and therefore the dangers of contamination are minimized. Bacteriological technique was used throughout.

Usually, for each condition tested, two different ages of cultures were used: "young" cultures (three or four days) and "old" cultures (seven or eight days). These cultures were started by introducing several thousand organisms by means of a platinum wire loop into 70 ml. of sterile proteose-peptone solution in a 125 cc. pyrex erlenmeyer flask.

After washing and after proper dilution or concentration (by centrifugation) 5 ml. of culture solution of known population density were added to each Barcroft-Warburg flask except to the thermobarometer which contained 5 ml. of buffered solution without organisms.

Oxygen consumption and CO_2 elimination were determined by the direct method. For this purpose 0.2 ml. 10 per cent KOH was put into the inner wells (insets) of one-half the flasks and 0.2 ml. H_2O in the other one-half. To each of the onsets was added 0.3 ml. 3N H_2SO_4 to absorb any ammonia that might possibly be formed in metabolism. In every case this acid was dumped into the main compartment of the flask at the end of an experiment after which a reading was always made in order to account for the bound CO_2 which is released by the action of acid.

Results

Relation between population density and O_2 consumption and CO_2 elimination

Tetrahymenas were obtained from cultures of different ages. "Young" cultures were produced in the following manner: about $2{,}000 \pm 200$ tetrahymenas were added to 70 cc. 2 per cent sterile proteose-peptone solution in pyrex erlenmeyer flasks. These cultures were kept at room temperature ($24° \pm 2°$ C.) for 3 or 4 days when the organisms were washed in the buffer solution [3] by centrifugation. They were then ready for testing. Several different population densities were used, ranging from approximately 10,000 to 195,000 organisms per ml. Several experiments were conducted for each density.

In these experiments 5 ml. of fluid containing young tetrahymenas were added to each of six Barcroft-Warburg flasks (3 with KOH in inner well; 3 with H_2O). Observations and readings were made from time to time during the course of the experiment. Usually readings were made every hour, but in the greater population densities, they had to be made every 15 or 30 minutes; whereas, with some of the lower population densities several hours elapsed in some cases before a final reading was made. It depended entirely upon the rapidity of oxygen consumption at 25° C. After the reading was made at the end of an experiment, the acid in the side arm of each flask was dumped into the fluid containing the tetrahymenas. The shaking apparatus was turned on for 10 minutes, after which time another reading was made. The results of these tests are presented in Table I.

The oxygen consumption per organism, when young cultures were used, is greatest in densities of 13,000 to 33,000 organisms per ml. which were the lowest densities used, and least in the greatest densities. In other words, at least within these limits, oxygen consumption per organism is inversely proportional to population density. The respiratory quotient varied from 0.95 to 1.13 in all the different tests, except those in which the lowest population densities were used where the R.Q. averaged 1.41.

The "old" organisms were produced in the same way as the "young" except that they were not used until 7 or 8 days after starting the cultures. The same procedures were followed as in the previous tests. The population densities varied between 12,000 and 265,000 organisms per ml. These results are also presented in Table I.

TABLE I

Oxygen consumption, carbon dioxide elimination and respiratory quotient in "young" and "old" cultures of Tetrahymena geleii *with different population densities. Temperature 25° C.; average volume of one million organisms = 23.6 mm.³; 42,370 organisms equal to one cubic millimeter.*

No. of organisms per ml.	Age of culture in days	No. of tests	O_2 consumption in mm.³ per hr. per million organisms	O_2 consumption in mm.³ per hr. per mm.³ cell substance	CO_2 elimination in mm.³ per hr. per million organisms	CO_2 elimination in mm.³ per hr. per mm.³ cell substance	R.Q.
Organisms from "young" cultures							
13,000–19,000	3	6	372	15.7	526	22.3	1.41
30,000–33,000	3	9	372	15.7	356	15.0	0.95
41,500–51,500	4	6	325	13.7	350	14.8	1.07
88,000–98,000	3	6	322	13.6	328	13.9	1.01
107,600–121,000	3½–4	9	252	10.6	249	10.5	0.99
195,000	3½	3	246	10.4	278	11.7	1.13
Organisms from "old" cultures							
12,000–12,600	7–8	9	198	8.4	221	9.3	1.11
24,000–27,500	7–8	6	223	9.4	258	10.9	1.15
38,000–39,400	7–8	9	204	8.6	247	10.4	1.21
69,000–72,600	7	6	278	11.7	357	15.1	1.28
87,600–95,000	7–8	6	217	9.2	263	11.1	1.21
135,000–148,700	7	9	207	8.7	240	10.1	1.15
213,000–265,000	7	6	230	9.7	272	11.5	1.18

The oxygen consumption varies from one population density to another much more so than in the "young" cultures, but there is a definite increase in consumption up to approximately 70,000 organisms per ml. and then a decrease with further increase in numbers per unit volume. Carbon dioxide elimination was also ascertained and the R.Q. was found to be greater than unity in every case where old cultures were used. The R.Q. increased progressively from 1.11 when approximately 12,000 organisms per cc. are used, to 1.28 when approximately 70,000 are used. It then decreases with further increase in cell population until it is 1.18 in cultures containing between 213,000 and 265,000 organisms per ml.

Relation between temperature and oxygen consumption

Tests were made to ascertain the oxygen consumption and carbon dioxide elimination in Tetrahymena at different temperatures. The temperatures used varied from 10° to 35° C. (with 5° increments). As in the previous experiments tests were run on both "young" and "old" organisms. The results are given in Table II.

There is an increase in oxygen cousumption as temperatures increase up to 25° C. Above 25° C. the O_2 consumption decreases directly with increase in temperature. This applies to organisms obtained from both young and old cultures. Attempts were made to ascertain oxygen consumption at 40° C. but the organisms died at this temperature. The carbon dioxide elimination and hence the R.Q. values were high in all the tests made. The latter ranged between 1.05 and 1.39.

TABLE II

The effect of temperature on oxygen consumption, carbon dioxide elimination and respiratory quotient in Tetrahymena geleii. *Average volume of one million organisms, 23.6 mm.³; 42,370 organisms equal to one cubic millimeter.*

Temperature, ° C.	No. of organisms per ml.	Age of culture in days	No. of tests	O_2 consumption in mm.³ per hr. per million organisms	O_2 consumption in mm.³ per hr. per mm.³ cell substance	CO_2 elimination in mm.³ per hr. per million organisms	CO_2 elimination in mm.³ per hr. per mm.³ cell substance	R.Q.
			Organisms from "young" cultures					
10	31,800	3	3	50	2.1	57	2.4	1.14
15	52,900–67,500	3–3½	9	125	5.3	142	6.0	1.13
20	38,750–41,160	3	6	193	8.1	227	9.6	1.17
25	41,500–51,600	4	6	325	13.7	350	14.8	1.07
30	32,000–60,000	3	6	227	9.6	297	12.5	1.30
35	10,000	3	3	215	9.1	287	12.1	1.33
			Organisms from "old" cultures					
10	54,000	8	3	25	1.0	35	1.5	1.39
15	53,000–73,300	7	12	92	3.9	91	3.8	0.99
20	65,500	6–7	6	128	5.4	135	5.7	1.05
25	69,000–72,600	7	6	278	11.7	357	15.1	1.28
30	45,000–50,800	7	6	228	9.6	285	12.0	1.25
35	16,800–19,160	8	6	254	10.7	287	12.1	1.12

O_2 consumption and CO_2 elimination in Tetrahymena in proteose-peptone solution

All the previous tests which have been reported here were made on Tetrahymena in the buffer solution given in footnote 3. None had been made on organisms in solutions in which they had grown and lived, i.e., proteose-peptone solution. The following experiment was carried out in order to make these determinations. Thus, the tests were conducted in the same way as the preceding ones, except that the organisms (1) were kept in the solution in which they had grown (2 per cent proteose-peptone) or (2) were washed in fresh proteose-peptone and then put into the manometer flasks in this solution. It was found that the hydrogen-ion concentration did not hold to a constant value as well as in the buffer solution but the change did not seem to harm the organisms (the greatest change noted was a change from pH 6.8 to pH 7.3). The results are presented in Table III.

In the tests in which "young" cultures of Tetrahymena were used in fresh proteose-peptone solution, the oxygen consumption proved to be much greater than in those tested in buffer solution (at 15° C., 271 mm.³ compared to 125 mm.³ per hour

per million organisms in buffer solution; at 25° C., 666 mm.^{3} compared to 325 mm.^{3}). In the organisms from "old" cultures, however, the oxygen consumption was somewhat lower than that in buffer solution (at 15°, 70 mm.^{3} as compared to 92 mm.^{3}). Variance in numbers, however, must be taken into consideration.

TABLE III

Oxygen consumption, carbon dioxide elimination and respiratory quotient in Tetrahymena geleii *in 2 per cent proteose-peptone solution. Temperature varied as indicated; average volume of one million organisms, 23.6 mm.^{3}; 42,370 organisms equal to one cubic millimeter.*

Temperature, °C.	No. of organisms per ml.	Age of culture in days	Condition of proteose-peptone solution	No. of tests	O_2 consumption in mm.^{3} per hr. per million organisms	O_2 consumption in mm.^{3} per hr. per mm.^{3} cell substance	CO_2 elimination in mm.^{3} per hr. per million organisms	CO_2 elimination in mm.^{3} per hr. per mm.^{3} cell substance	R.Q.
15	30,400	3	fresh	4	271	11.4	328	13.9	1.21
15	32,060–40,000	7	old	6	70	2.9	197	8.3	2.81
25	25,800	3	fresh	3	666	28.2	849	35.9	1.27

A very interesting observation brought out by these results, is that in all tests, the R.Q. is considerably above 1.0. This is especially so in the case of the "old" organisms where the average R.Q. for all tests was 2.81.

Significance of the high respiratory quotients

The question arises as to the true meaning of these high respiratory quotients. In all but a few experiments covered by this report, they are greater than 1.0. It is generally known that high values for R.Q. may be obtained chiefly under two conditions: (1) when fat is in the process of formation in cells or tissues, or (2) when it is necessary for the cell or tissue to go into oxygen debt, temporarily at least, during which time the ratio between CO_2 eliminated and O_2 consumed would be much greater than usual.

Tetrahymena produces fat in the form of small globules located in the anterior half of the organism. Observations were made upon these organisms under various conditions in order to ascertain whether or not respiratory quotients varied and if so, whether or not fat content varied also. As far as could be seen by the methods used there was no difference in fat content in organisms under different conditions even though the respiratory quotients varied considerably.

A few tests were made in an attempt to ascertain the oxygen consumption and carbon dioxide elimination of *Tetrahymena* in nitrogen. The nitrogen used, however, was a commercial grade and hence traces of oxygen were present. Although the results obtained by these tests were not conclusive, they indicate that these organisms are anaerobic, at least to a limited extent, thus confirming the results of Thomas (1942). The respiratory quotients calculated from the results ranged between 1.49 and 2.87.

DISCUSSION

Hall (1938, 1941), Baker and Baumberger (1941) and Ormsbee (1942) have studied respiration in *Tetrahymena geleii*. The results of these several investiga-

tions appear to differ, in some cases, considerably but when they are analyzed carefully the differences, for the most part, may be explained.

Some of the results of the present investigation are in close agreement with those of the aforementioned workers. This is especially true, when our results are compared with those of Ormsbee (1942) in which he found that the O_2 consumption for these organisms in 2 per cent proteose-peptone solution is 632.5 mm.^{3} per hour per million at 26.8° C.; in our studies, it was found that the O_2 consumption in 2 per cent proteose-peptone is 666 mm.^{3} at 25° C. The results obtained in non-nutrient media should not be compared since the salts differ greatly in the solutions used by different investigators, but it is found that even in these cases there is some agreement, especially when all the factors are taken into consideration. An explanation of some of the differences, for example, might be found in the fact that different population densities were used. According to our results, as the density of population increases the rate of oxygen consumption per individual decreases. This observation, along with possible differences in age of cultures, and also the difference in culture media before and during the experiment, could account for the slight difference in oxygen consumption as noted in these reports. This age difference is especially noticeable when the organisms are tested in the proteose-peptone solution in which they were grown. There is not only an inhibition of division with age but also a great decrease in oxidative metabolism.

Thomas (1942) showed definitely that *Tetrahymena* is adapted, at least partially, to an anaerobic existence, recovery being dependent upon oxidative metabolism. He believes the processes involved may be similar to those of mammalian striated muscle. Pace and Ireland (1945) showed that *Tetrahymena* can live and grow at very low oxygen tensions but that its growth is best at high tensions. This organism does not grow or live for any great length of time in the total absence of oxygen. Results presented in this report confirm those of Thomas in this respect.

The respiratory quotients obtained for *Tetrahymena* were all high under the conditions in which they were determined. They were highest of all in those organisms confined in practically pure nitrogen. Carbon dioxide is produced in large quantities. It is possible that the metabolism is similar to that of vertebrate striated muscle. The extent of anaerobiosis is limited for *Tetrahymena* just as it is for muscle. *Tetrahymena* can live for several days in the absence of oxygen but soon dies unless oxygen is added. Specht (1934) found the same to be true for *Spirostomum*. He obtained high R.Q. values when this organism was exposed to low O_2 tensions. Many protozoan forms function in the same way (von Brand, 1946).

Summary

1. Oxygen consumption and carbon dioxide elimination of *Tetrahymena geleii* has been ascertained for different temperatures, for different population densities, and for "young" and "old" cultures.

2. The tests were conducted in most of the experiments with washed organisms in inorganic buffer solution.

3. When "young" organisms are used, the oxygen consumption per unit volume of cell substance is inversely proportional to population density. The respiratory quotients for all tests were nearly always above 1.0.

4. When "old" organisms are used there is an increase in O_2 consumption per unit volume of cell substance with an increase in population density up to 69,000–

72,600 organisms per ml. Densities above this result in lower consumptions. Respiratory quotients varied from an average of 1.11 in low population densities to 1.28 at optimum densities.

5. Oxygen consumption is always greater in the "young" cultures than in the "old" cultures, densities being equal.

6. With an increase in temperature, the O_2 consumption increases to a maximum at 25° C. in both "young" and "old" cultures. Above this temperature the consumption decreases. In all tests, from 10 to 35° C., R.Q. values were above 1.0.

7. "Young" organisms, tested in fresh solution of the same kind in which they were grown, 2 per cent proteose-peptone (Difco), show much greater O_2 consumption than those tested in inorganic buffer solution. "Old" organisms, however, tested in the same solution in which they had grown, have a lower O_2 consumption than old specimens tested in fresh inorganic buffer solution.

8. Very high respiratory quotients were obtained for the organisms in proteose-peptone, especially in "old" cultures where the average was 2.81.

9. In nitrogen gas with but minute traces of oxygen, *Tetrahymena* utilizes the small quantity present and gives off comparatively large quantities of carbon dioxide. The R.Q. values are extremely high.

10. *Tetrahymena geleii* is to a limited extent anaerobic but it can live only with difficulty for more than a few days in the absence of oxygen.

LITERATURE CITED

Baker, E. G. S., and J. P. Baumberger, 1941. The respiratory rate and the cytochrome content of a ciliate protozoan (Tetrahymena geleii). *J. Cell. Comp. Physiol.*, **17**: 285–303.

Hall, R. H., 1938. The oxygen consumption of Colpidium campylum. *Biol. Bull.*, **75**: 395–408.

Hall, R. H., 1941. The effect of cyanide on oxygen consumption of Colpidium campylum. *Physiol. Zool.*, **14**: 193–208.

Ormsbee, R. A., 1942. The normal growth and respiration of Tetrahymena geleii. *Biol. Bull.*, **82**: 423–437.

Pace, D. M., and W. H. Belda, 1944. Effect of food content and temperature on respiration in Pelomyxa carolinensis Wilson. *Biol. Bull.*, **86**: 146–153.

Pace, D. M., and R. L. Ireland, 1945. The effects of oxygen, carbon dioxide, and pressure on growth in Chilomonas paramecium and Tetrahymena geleii Furgason. *Jour. Gen. Physiol.*, **28**: 547–557.

Specht, H., 1934. Aerobic respiration in Spirostomum ambiguum and the production of ammonia. *Jour. Cell. Comp. Physiol.*, **5**: 319–333.

Thomas, J. O., 1942. The anaerobic carbohydrate metabolism of Tetrahymena geleii. *Dissertation, Stanford University.*

von Brand, T., 1946. Anaerobiosis in invertebrates. *No. 4 of the Biodynamica monographs edited by B. J. Luyet, Normandy, Mo.*